Indian Tea Association.

THE

FERMENT OF THE TEA LEAF,

AND ITS RELATION TO QUALITY IN TEA.

BY

HAROLD H. MANN, B. Sc.,

SCIENTIFIC OFFICER TO THE INDIAN TEA ASSOCIATION.

CALCUTTA:

PRINTED BY W. NEWMAN & CO.,

AT THE CAXTON STEAM PRINTING WORKS, 1, MISSION ROW.

1901.

BIBL. DU MUSEUM PARIS

Bon bibliothèque d'ethnobiologie N° 30614

264007

Indian Tea Association.

THE

FERMENT OF THE TEA LEAF,

AND ITS RELATION TO QUALITY IN TEA.

BY

HAROLD H. MANN, B. Sc.,

SCIENTIFIC OFFICER TO THE INDIAN TEA ASSOCIATION.

BIBL. DU MUSEUM PARIS

CALCUTTA:

PRINTED BY W. NEWMAN & CO.,

AT THE CAXTON STEAM PRINTING WORKS, 1, MISSION ROW.

1901.

BIBL. MUSEUM PARIS

THE

FERMENT OF THE TEA LEAF,

AND ITS RELATION TO QUALITY IN TEA.

From the commencement of the manufacture of Black Tea in India the nature of the process of fermentation, which plays such an important and essential part in the manufacture of tea, has been a subject of controversy. In the years before 1880 for instance, a most energetic discussion took place.* On the one hand it was contended, and with good show of reason, that the process was merely one of incipient putrefaction. On the other, with equal vigour and with equal reasonableness, that the result obtained was entirely independent of any tendency to rot, and, though it might run parallel with it, was an entirely independent process. Inasmuch as all these views were based entirely on speculation, and were put forth without an attempt even at a microscopic examination of fermenting leaf, they naturally led to no better understanding of the question.

MR. BAMBER'S EXPERIMENTS.

The investigations of Mr. Bamber were, in fact, the first of any importance on the subject, and he has remained practically ever since the standard, if not the only, authority on the subject. His investigations, which it is well to recall, led him to make certain experiments, the following account of which I quote from his "Chemistry and Agriculture of Tea." †

"The following experiments were made in Assam to determine whether the changes, which take place during the process, were due to the presence of either an organised or soluble ferment or merely to oxidation:—

1st.—Freshly rolled leaf was placed under a receiver, and the air exhausted as completely as possible. Little or no change took place, and the leaf at the end of 24 hours was still a dull green colour, with a little brown colour on the stems. Admission of air or oxygen at this period had no effect on the colour, probably owing to the comparatively dry condition of the leaf.

---

* See Tea Cyclopædia, p. 211. † Calcutta 1893.

2nd.—In this experiment the air was exhausted as before, and pure dry oxygen gas admitted; in half an hour the leaf had attained a bright coppery colour, and in two hours the whole of the leaf was a uniform dark red, and had gone beyond the stage required for ordinary manufacture. Duplicate experiments were made of the above with similar results, and in each case a asmple of the same leaf was treated in the usual manner for comparison with the experimental leaf, the flavour of the teas treated with oxygen gas did not differ from the ordinary teas, but the infusion had a brighter appearance.

3rd.—The rolled leaf was treated in vacuo with pure carbonic acid, which is an inert gas, with the result that at the end of 5½ hours it was still a dirty green colour, while a sample treated in the usual manner attained the required colour and condition in 3 hours.

4th.—The leaf was exposed to a limited supply of air and even after 20 hours was still of a greenish colour, and the tea manufactured from it was very pungent, showing that little of the astringent properties had been destroyed.

5th.—The rolled leaf was treated with dry steam at a high temperature for a few minutes, and was then treated as usual, when it attained a good bright colour.

6th.—The rolled leaf was treated with air and oxygen gas, which gave a bright colour the liquor was flavoury, but not very pungent, and infusion bright.

All the above experiments tend to show that the change in the leaf in the so-called 'fermentation' is due to oxidation. Microscopic examination has failed to show any organism; and the fact that the change will take place in an hour or less from the breaking of the cells is, I think, conclusive evidence that it cannot be due to the development of living organisms."

These experiments, thus reported, formed an enormous advance on the preceding condition of ignorance on the question. But yet on careful examination it will be seen that Mr. Bamber's conclusions were not quite justified by his experiments. In one case he has since* withdrawn from the position he then took up, and has himself announced the discovery of an oxidase or soluble ferment to which he now ascribes the changes occurring during fermentation. He announced this last discovery in the following words :—

"Quite recently I have succeeded after numerous attempts in isolating a minute proportion of a soluble oxidising ferment, somewhat similar to the oxidases recently discovered in several plants of different natural orders. The substance in question, which evidently has a considerable hearing on the oxidising properties of the tea, apparently does not exist in the active form in the fresh green leaf, but is changed either during the withering, if the leaf is bruised, or during the rolling processes when the various organic acids, etc., are liberated from the cells.

The discovery here made has been confirmed by one or two independent observers since the time. †

In another point Mr. Bamber was also in error in the views expressed in 1893. He states that he found no living bacteria or

* Report on Ceylon Tea Soils, Colombo 1900. † See Also Bull. Roy. Agric. College, Tokio, 1901, &c.

other organisms on the fermenting leaf, but I have never been able to find tea during this process on which a comparatively large number of organisms could not be found. Whether they have any part in the process is another question, but they are in evidence on every sample which I have examined.

FERMENTATION AT A HIGH TEMPERATURE.

But assuming that the conclusions are in general correct, certain results would naturally follow. It would seem likely that a high temperature would be the best and quickest for fermentation, since oxygen is then most active and the action of stray microbes would be entirely eliminated above a temperature of 110° F. It therefore seemed advisable to try whether fermentation would proceed normally and correctly if the mass were raised above that temperature. Two samples of rolled tea leaf were therefore taken, one fermented at ordinary temperature (73°-74° F.), and the other, with due precautions that no drying of the leaf took place, at 120° F. As a matter of fact to all appearance, both coloured normally, that at the higher temperature a little more rapidly. When apparently ready both samples were fired in the same way, and the tea examined. In the result it was found that the hot fermentation gave a slightly lighter, but a little duller liquor. It had a tendency, though not a very marked one in the present case, to be soft to the taste.

So far, however, the result is in conformity with the idea that bacterial action could have nothing to do with the operation, seeing that no organisms could be active at the temperature employed, and yet a fermentation, not quite normal, certainly, but nearly so, could be carried out.

OXIDATION BY CHEMICALS.

It might, however (still on the basis of the above assumption that oxidation by contact with oxygen gas is all that takes place), be possible to assist the action by some substance energetic in giving oxygen to any body with which it was mixed. Two such substances, both of which could not damage the tea were therefore tried. These were Hydrogen Peroxide and Permanganate of Potash, either of which could be bought, were a demand to arise, at a comparatively cheap rate.

With Hydrogen Peroxide 4 ozs. of a "one volume" solution was added to 2 lbs. of leaf just from the roller, and fermentation allowed

to proceed. After 45 minutes the lot of leaf treated was distinctly brighter in colour. The fermentation was considered complete in each case after 1½ hours, and both were dried in the same manner. I sent the teas to an expert taster who was good enough to make the following report:—"I give preference to 'B' (without Hydrogen Peroxide) samples which have much better appearance than 'A' (with Hydrogen Peroxide), and have more body. On the other hand 'A' samples have fine Darjeeling flavour, and almost perfect infusions. They only want a little more body in the cup, and less greyness in the dry leaf to make them very desirable teas." Eliminating all reference to the appearance of the dry leaf, it appears that the addition of an oxidising agent had little effect on the quality. A repetition of the experiment gave the same result.

In similar manner Permanganate of Potash was added to another lot of rolled leaf (4 ozs. of 1 per cent. solution to 3 lbs. of leaf) and on drying after being fermented for the same time no difference could be detected in color of liquor, taste, or pungency.

The mere addition of Oxygen in an active form was therefore not capable of making the fermentation go either perceptibly more quickly, or more evenly. This would afford, were it needed, strong evidence against any theory of mere oxidation as explanatory of the changes which undoubtedly take place.

BACTERIA IN TEA FERMENTATION.

Though the first experiment above described appeared to have eliminated the possibility of bacteria being the cause of the changes, another effort was made to isolate such as were present, and likely to be of any importance, so that one might then ascertain whether they had any influence on the fermentation of rolled tea leaf. This was done by grinding up some fresh tea leaf to a pulp, then sterilising it by means of steam, and finally introducing into it a piece of fermenting tea leaf. After two days it was evident that growth of some organism was taking place, and on examination this was found to be a small rod-shaped microbe. I only succeeded in isolating this one type of organism from all the cultures that I made, but it was nevertheless invariably present. After a second culture in tea juice, freshly rolled tea was inoculated with this microbe, and allowed to ferment. After 1½ hours the tea was sour, while a check lot of rolled leaf to which nothing had been added was fermenting normally. This, I think, therefore, finally sets at rest the question as to whether

actual living organisms play a part in the ordinary fermentation of tea. Though they are present always, they are present as impurities, and have nothing to do with the action which it is desired should go on, and they should be eliminated, so far as is possible, from the fermenting leaf, and the fermenting house.

FERMENT OF THE TEA LEAF.

But nevertheless there must be some active cause in the tea leaf which causes the production of the desired colour and flavour, especially as we have shown that oxygen alone is not capable of producing the effect. And this cause is the presence of a representative of a class of ferments—not actually living but always produced by living cells,—the discovery of which in tea was, for the first time, as stated above, made by Mr. Bamber in the early part of 1900. These "unorganised ferments" or "enzymes" as they are termed have been discovered in recent years to play a most important part in many operations where their presence previously had been hardly suspected. Taking the "oxidases," for instance, the class of enzymes which is active in the case of tea, it has been shown that ferments of a similar type also take a principal part in the production of Japanese lacquer,* and further, as has been still more recently discovered, in the curing and fermentation of tobacco. †

EXTRACTION OF THE FERMENT.

Their discovery in the present instance was doubtless much delayed by the presence of the large amount of tannin in the tea leaf, which makes it difficult, if not impossible, to extract the ferment unless special means are used to previously remove this tannin. My final method was as follows:—A known quantity of leaf (10 grams of fresh or 6·6 grams of withered) was ground up to a pulp, and then hide powder (5 grams) was added and the mixture thoroughly ground together. This hide powder has the faculty of removing the tannin from the liquid in which it is present. When a known quantity of water was added, therefore, the tannin having been removed from solution by the hide powder, the enzyme was allowed to exude into the liquid. After standing for two hours the whole mass was pressed through cloth, and all the extractable part, at any rate, of the ferment was obtained in the liquid thus squeezed out. On the addition of alcohol the enzyme was deposited as a slimy mass,

* Bertrand, 1894 and 1895.

† Oscar Loew. Bulletins of the United States Department of Agriculture, 1899-1901.

which on being again dissolved in water and filtered, gave a clear liquid containing the ferment required. If it was wished to determine its quantity use was made of the fact that on addition of "guaiacum resin tincture" a blue colour was produced, and by measuring the intensity of this blue colour the relative quantity in two similarly prepared solutions could be ascertained. Further it was found that all the enzyme thus extracted was by no means equally active, and that it could be divided into two parts one of which gave the blue oxidation product with "guaiacum resin" alone, and the other only in presence of a small amount of hydrogen peroxide. In this report I have called the former of these "active enzyme," and the whole present, including the latter, "total enzyme," though probably a part of this does not exist ready formed in the leaf, and may hence be known as "zymogen" or "pro-enzyme."

OXIDISING ACTION OF THE FERMENT.

After isolating these substances the first question would naturally be as to what effect they have when added to fermenting tea leaf. The experiment was therefore made, and it was found that the leaf coloured more quickly in their presence and at the same time the colouring proceeded normally. The same happened if tea juice instead of tea leaf was taken. It may therefore be taken that the ferment which I have isolated does play a part in the fermentation of the tea. This was confirmed by its action on several other easily oxidisable substances. Pyrogallic Acid, for instance, coloured (*i. e.*, oxidised) with great rapidity in its presence. Hydroquinone did the same. So that we have here an oxidising enzyme or oxidase, present in the tea leaf, capable of causing increased rapidity of fermentation in the tea, and capable of oxidising various easily oxidisable substances like Pyrogallic Acid and Hydroquinone.

EFFECT OF HEAT, &c., ON THE FERMENT.

The next point appeared to be to ascertain the effect of heat upon this oxidase, especially as this has an important bearing on the temperature necessary to stop the fermentation at the commencement of firing. It was found that a temperature of 160-165°F. had no appreciable effect in three minutes, that at 176°F. the oxidase was partly destroyed on the same time, but it was not till over 180°F. was reached that the ferment was completely destroyed in this period. On the other hand its activity is considerably

modified at a much lower temperature. I could not ascertain the precise point at which it ceased to be effective, but while very active at 130°F., it was much more feeble at 143°F., and doubtless at a very short distance above this it ceases altogether to exercise its functions. It is therefore absolutely necessary that the whole of the tea in the drying machine (and not merely the machine thermometer, which is a very different thing) be raised considerably above 150°F. immediately on putting in the firing machine, and to ensure the destruction of the ferment at least 180°F. should be reached. This is of course what is attained in the best practice, but the reason and necessity for it become obvious when considered in the light of the above observations.

The effect of various chemicals on the oxidase was next considered. It acts best in slightly acid solution, but if this acidity be increased especially with mineral acids, beyond a small amount, all action ceases and the enzyme may be destroyed. ·4 per cent. of Sulphuric Acid in a liquid was sufficient to absolutely destroy the ferment at once. ·04 per cent. apparently hindered all oxidising action. To organic acids such as those which occur in tea juice it was much less sensitive, and 3 per cent. of Acetic Acid were required to destroy it in 2 hours. Alkalies were less effective, but here 3 per cent. of Ammonia or Caustic Potash were sufficient to practically destroy the active part of the enzyme after four and a half hours. The result in manufacture of these observations I have not as yet been able to ascertain. Whether it may be wise or practicable to make up the leaf to a certain definite acidity, being the point at which the ferment was most active, before fermentation, one cannot at present say. It is well known that Hydrochloric Acid added to the roll softens and weakens the liquor,* but in this case the acid itself is objectionable, and we have no record of the amount added. This is a matter for experiment.

DISTRIBUTION OF FERMENT IN THE FLUSHING SHOOT.

If the various leaves on a flushing shoot be taken, the amount of enzyme is by no means the same in every part. The fresh leaf, for instance, contains about an equal amount in the unopened tip leaf and in the stalk, but below the tip the percentage decreases in every leaf. Taking the leaf as plucked, for instance, on a China-hybrid bush in September, the following table gives the relative

* See Tea Notes. A. F. Dowling, 1885.

amount present in each leaf separately, calculated both on the fresh leaf and on the dry matter in the leaves (taking that in the tip leaf as unity):—

	Active Enzyme.		Total Enzyme.	
	On fresh leaf.	On dry leaf.	On fresh leaf.	On dry leaf.
Unopened tip leaf ...	1·00	1·00	1·00	1·00
First open leaf ...	·64	·65	·64	·61
Second open leaf ...	·48	·48	(·80 ?)	(·80 ?)
Stalk ...	1·13	1·64	·95	1·39

These figures apparently seem to indicate that where the largest quantity of enzyme is present, the best tea is made, and yet not wholly so, because the stalk, which is objectionable in the tea, contains as much as any part. The reason of this is seen however if the relative amount of acidity, of tannin, and of phosphoric acids in the same samples of these leaves are taken. These give the following figures:—

	Acidity.		Tannin.		Phosphoric Acid.	
	In fresh leaf.	In dry leaf.	In fresh leaf.	In dry leaf.	In fresh leaf.	In dry leaf.
Tip unopened leaf ...	1·00	1·00	1·00	1·00	1·00	1·00
First open leaf ...	94	·94	1·03	1·03	·88	·88
Second open leaf ...	·94	·94	·91	·91	·75	·75
Stalk ...	·47	·70	·59	·86	·55	·79

It therefore appears that, where a large amount of enzyme is combined with the greatest acidity, and with the greatest amount of tannin, there the tea produced is the best. Such is only a preliminary conclusion, and it must strictly be considered applicable to similar conditions. It is, however, one to which the next set of experiments gives support.

RELATION OF FERMENT TO QUALITY.

Several gardens were taken in the Darjeeling District. A produces average or rather better than average Darjeeling tea; B has for many years produced absolutely the best tea in India;

C is during the present season giving the highest priced product in the district. Conditions being therefore as near as possible equal, the quality, if the above contention be true, should vary according to the amount of enzyme present, pr ovided the same amount of stalk, or approximately so, be present in the samples. Comparing, first, garden B with garden A. B No. 1 is leaf from a young Assam or high hybrid extension giving very fine tea ; B No. 2 is from a low level Assam extension giving the worst tea on the garden, but yet an above-average quality ; B No. 3 is from China tea giving an excellently flavoured product. Determining the enzyme present in each of these samples in September 1900, and comparing the amount with that in A (China hybrid plant), we have, taking A as unity.

	Active Enzyme.	Total Enzyme.
A	1·00	1·00
B No. 1	1·88	1·30
B No. 2	1·17	1·32
B No. 3	1·83	1·32

In this case the active enzyme seems therefore to be a fair measure of the quality-producing character of the leaf. The same result is shown on garden C, as follows :—

	Active Enzyme.	Total Enzyme.
A	1·00	1·00
C No. 1	2·17	2·18
C No. 2	1·44	1·68

Here C No. 1 represents the very highest quality Assam bushes, and C No. 2, similarly, the best China plants in the garden. In C No. 1, probably a little larger amount of stalk occurred, but A and C No. 2 are absolutely comparable, and here it will again be seen that flavour in the tea follows the enzyme in the leaf. Hence one may, I think, conclude that, other things being equal, the flavour in the product is materially connected with the quantity of oxidase in the leaf from which is made. This conclusion, as stated above, will have to be supported by many more experiments before one can consider it satisfactorily established, but in the meantime there is strong and consistent evidence of its substantial accuracy.

BIBL. DU MUSEUM PARIS

How then can this oxidase be increased in the leaf? In a table on page 8, it was shown that, taking the various leaves on the same stalk, the amount of phosphoric acid varied very closely with the amount of oxidase. In addition to this I have, in a previous report,* brought forward very strong evidence that the "quality" of tea is materially influenced, at any rate in Assam, by the amount of phosphoric acid, and especially of available phosphoric acid, in the soil. Now not only is phosphoric acid present in greater quantity in the leaves on the same stalk which give the most enzyme and produce the best tea, but also there appears to be most of this constituent in the soil of those gardens giving leaf containing the most oxidase, and making the best tea. The following figures for the soil of the gardens A and C, where the leaf mentioned above was obtained, show this very clearly.

	A	C
Percentage of Phosphoric Acid in Soil.	·061	·124

The conclusion drawn in my previous report above mentioned, that in order to obtain high quality tea, there must in any case be a large quantity of phosphoric acid present in the soil, is here confirmed, and this phosphoric acid becomes, in addition, apparently connected with the quantity of enzyme in the tea leaf.

INCREASE OF FERMENT DURING WITHERING.

One method by which the amount of oxidase present in the leaf may be, and is normally, increased, must not go by unnoticed. Withering has usually been considered to mean partial drying of the leaf at a low temperature so as to make it fit for rolling—and no more. My experiments show, however, that it has a far more important function, which may explain to a certain extent the failure of artificial methods by artificial heat to give a wither of the best kind. During this process, in fact, the amount of active ferment materially increases, in some cases by as much as 80 per cent. of its original amount. Taking the leaf from gardens A and B above (page 9), the following increase took place during withering, due allowance having been made for the loss in water

* Tea Soils of Assam and Tea Manuring. November 1901.

during the process, and taking the amount in the fresh leaf from Garden A as unity.

	Active Enzyme.		Total Enzyme.	
	In fresh leaf.	In withered leaf.	In fresh leaf.	In withered leaf.
A	1·00	1·81	1·00	1·69
B No. 1 ...	1·88	2·48	1·30	1·87
B No. 2	1·17	1·88	1·32	1·87
B No. 3	1·83	2·19	1·32	2·19

The increase in active ferment hence varies from 20 per cent. to 80 per cent. of the amount originally present, and this accounts very well for the fact that leaf rolled fresh without withering will never colour like properly withered leaf.

CONCLUSIONS.

What practical conclusions can therefore be drawn from the investigations which have just been described? Will it be possible to prepare this enzyme, and at a time when the quality of the tea is inevitably low to add it to the fermenting leaf in order to improve the flavour? This may be so, but is, to say the least of it, very doubtful. It must always be remembered in discussing the value of the presence of any ferment whatever, that it is not only the ferment which is of importance, but also the substances to be fermented. And in all vegetable products the growth of the material to be changed is coincident with that of the enzyme which is to change it. This is, for instance, the case in leaves or in seeds containing starch. So soon as starch appears, there also appears the diastase which is to transform it into sugar. This is the same with other ferments and fermentable substances. It would therefore not seem likely at first sight that by merely adding the ferment alone from outside any great improvement would be effected. But, so far, we are in ignorance what the substance is by which flavour is produced. The property may reside in the tannic acid, but this there is every reason to doubt, though of course to that constituent we do owe undoubtedly the pungency of the tea. It may be due to some changes in the resins, in the pectins, or in other products of the leaf of more or less obscure character. What these are, and how they are changed will forms the next step in this investigation.

For the present it has been established—

(1). That the fermentation of tea is independent of living organisms, and is caused by an unorganised ferment or oxidase which can be extracted from fresh or withered leaf.

(2). That the quantity of this oxidase is greatest in the tip leaf of the shoot, and becomes less and less in the leaves as one descends from the growing point. It occurs in two forms, one more active than the other.

(3). That the oxidase acts best in a slightly acid juice, but excess of acid destroys its power, as well as any free alkali in the liquid in which it works.

(4). That it is materially increased during withering, and hence this process has a very important function in tea manufacture, independent of its necessity in making the leaf fit to roll.

(5). That, given leaf of the same character, the quality of the tea produced varies with the amount of oxidase, the larger the amount of oxidase the better being usually the quality of the tea.

(6). That the amount of oxidase in the leaf is, in some way not yet known, dependent on the amount of phosphoric acid in the soil.

The work here detailed was done principally on the Moondakotee Tea Estate, Darjeeling, and to the manager, the agents, and the owners of that garden, I must here tender my heartiest thanks for help in every direction. To the Reporter on Economic Products to the Government of India, my thanks are also due for the use of his Calcutta laboratory in connection with this work.

BIBL. MUSEUM PARIS

[600—9-12-1901.]